

## STRUCTURE AND FUNCTION OF THE TERMINAL ABDOMINAL APPENDAGES (PYGYPODIA) OF PHOTURID FIREFLY LARVAE

PETER DOMAGALA AND H. GHIRADELLA

*Department of Biological Sciences, State University of New York at Albany, Albany, New York 12222*

### ABSTRACT

The morphology of the pygypodia, the eversible terminal appendages of the abdomens of photurid firefly larvae, was studied with particular reference to the integumental and muscle systems. The eversible nature and observed versatility of these appendages apparently result from the antagonistic action of pygypodia-associated muscles and those muscles which control (pseudo)coelomic fluid pressure in the abdomen, as well as from several fine structural features of the integument. Continuous sheets of parallel microfibrils in the endocuticle form a tough elastic inner sheath within each pygypodium. The integument bears rows of spurs, whose interconnection by the microfibril sheets may aid in their positioning as well as in their orderly and consistent packing during inversion and eversion of the pygypodia. The loose organization, irregularity of shape, and sparsity of sites of attachment of the epidermal cells, relative to the basement membrane, may all contribute to the flexible, yet strong, inner structure of the integument.

### INTRODUCTION

Much is known about the anatomy and function of typical larval insect abdominal segments and their appendages (see Snodgrass, 1935, for review). There is little information, however, about less usual appendages such as the eversible pygypodia (Snodgrass, 1935) on the terminal abdominal segments of photurid firefly larvae. These appendages are extremely versatile in enabling the animal to attach to and move about on a variety of substrates. Their versatility and relatively simple structure makes this an interesting system, both in its basic morphology and in the opportunity to relate this morphology to the observed functions.

This paper describes the gross anatomy and the fine structure of these photurid pygypodia with particular reference to the integument and muscle systems. We also consider the functions of these appendages and speculate on their possible evolutionary origins.

### MATERIALS AND METHODS

*Photuris* larvae were collected in Guilderland (near Albany, New York) during May and July and were stored cold and damp, in a controlled light/dark regime (McLean *et al.*, 1972). The larvae were fed crushed snails and were maintained in an intermolt state.

A variety of light and electron microscopical techniques, including bright field, phase, and Nomarski differential interference microscopy, and transmission and scanning electron microscopy, were used to observe dissected specimens and whole mounts of the pygypodia. To maintain the pygypodia partially or completely everted, larvae

were injected with water-insoluble Vinyl Acetate Red (Ward's Natural Science Establishment). This compound polymerizes into a rubbery mass which plugs the pygypodia and keeps them from collapsing or being retracted.

For transmission electron microscopy (TEM), individual pygypodia were dissected out and immediately fixed (two hours; 4°C in 0.1 *M* cacodylate-buffered 3% glutaraldehyde at pH 7.2). The tissues were rinsed in buffer and post-fixed overnight (4°C in 1% osmium tetroxide in 0.1 *M* cacodylate buffer). All tissues were dehydrated in a graded ethanol series and were embedded in Spurr low viscosity resin (Spurr, 1969). Thin sections were cut with glass knives or with a Dupont diamond knife on an LKB Ultratome III. The sections were mounted on 100 or 200 mesh uncoated grids and were stained for 25 minutes with saturated uranyl acetate in 50% methanol at 37°C, and for five minutes at room temperature in lead citrate (Reynolds, 1963).

The specimens were examined at 60 kV with an AEI EM6B transmission electron microscope, which was calibrated by a #10002X crossed-line grating replica calibration grid (E. F. Fullam, Inc.).

To study cuticular elements, pygypodia were split longitudinally, dissected out, and placed for a week in a 10% solution of aqueous potassium hydroxide, which dissolves the cellular elements and extracts some of the cuticular structures, but leaves the exocuticle essentially intact.

For scanning electron microscopic (SEM) observation, pygypodia were everted by the vinyl acetate treatment, air dried, and then sputter-coated with gold and observed in an ISI Super Mini II scanning electron microscope.

## RESULTS

An undisturbed larva typically rests with its pygypodia everted or extended (Fig. 1). When the animal walks, it inverts or retracts the entire pygypodial apparatus, using the blunt end of the abdomen as an "appendage" accessory to the legs. In this way, the abdomen both supports the body and provides a point of anchorage or adhesion against which the rest of the animal's locomotory apparatus can work.

Under special conditions, *i.e.*, hanging from an edge or righting itself when it has been turned on its back, the animal everts the pygypodia, which then provide a stable site of attachment and free the rest of the body to explore or to otherwise respond to the particular situation. This attachment is equally effective on rough (*e.g.*, paper, soil, etc.) or smooth (*e.g.*, glass or plastic) substrates.

Control of pygypodial movement appears selective; not all the podia necessarily evert or invert at a given time, nor need all extend or retract to the same degree. All in all, the pygypodia are quite effective in accommodating to whatever substrates they encounter.

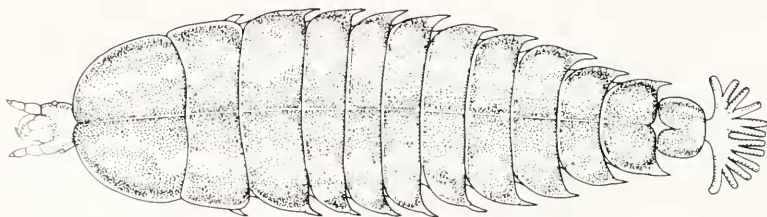


FIGURE 1. Dorsal view of a mature photurid larva. The everted pygypodia form the fan-like structure emerging from the rear.

### *External morphology*

The pygypodial apparatus is located on, and can be withdrawn into, the ninth abdominal segment (Figs. 1, 2) and an extension thereof. Because of the small size of the extension, it is difficult to determine if it is actually a tenth abdominal segment. Dissection reveals that the extension appears to have lost the tergal plate, being essentially covered by the tergum of the ninth segment, but that it does have pleural plates and a small and indistinct sternum.

All pygypodia originate from a common basal stalk (Figs. 1, 2a) which is continuous with the flexible integument of this last abdominal segment, the extension of segment IX. The entire pygypodial apparatus exhibits bilateral symmetry; each side consists of eight individual podia, of which six are located dorsally and two ventrally. Of the dorsal six, the outer four originate from a common branch of the main stalk, whereas the remaining two come from a separate, more medial branch (Fig. 2a). Each ventral set of two pygypodia originates from yet another branch of the main stalk. Together, when everted, the twelve dorsal pygypodia display a fan-shaped pattern (Figs. 1, 2a), and the ventral pygypodia assume a smaller, but similar pattern.

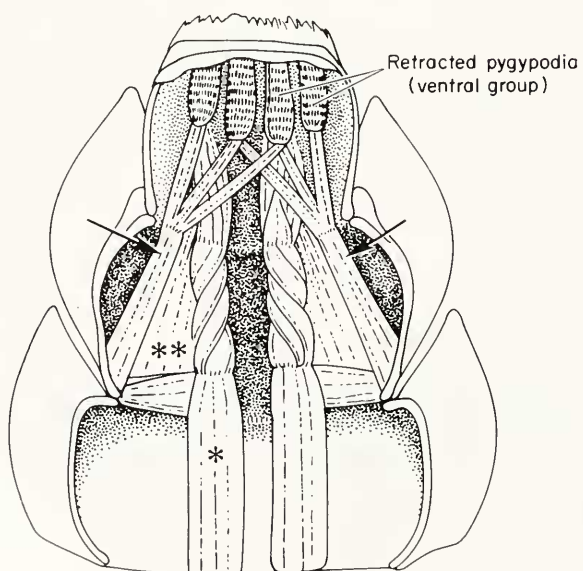
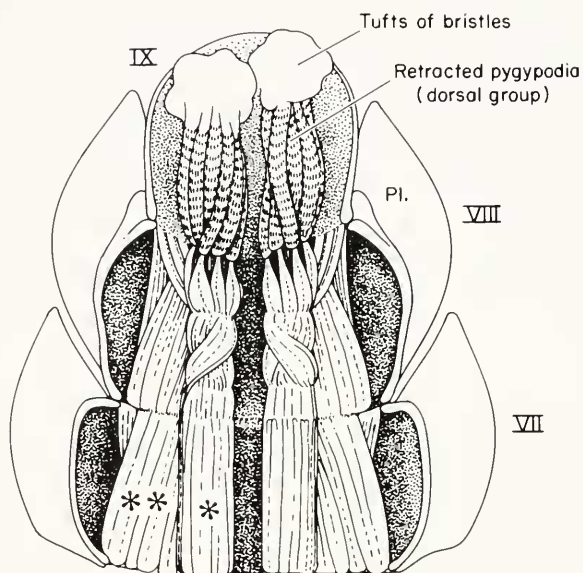
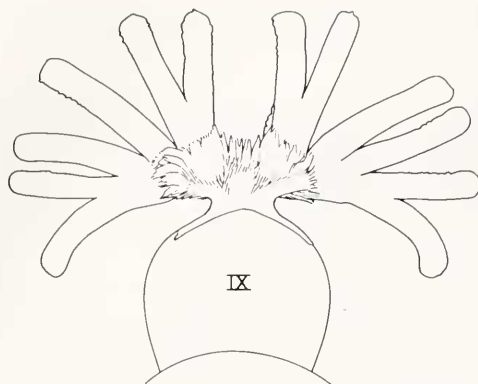
Individual pygypodia are cylindrical, and each has a longitudinal crease running dorsally along the entire length and a distinct protrusion or bulb at the tip (Fig. 4). The more medial podia are slightly longer than the lateral ones, and the ventral podia are shorter and stouter than their dorsal counterparts.

The pygypodia have on their surfaces sets of equidistantly-spaced transverse rows of cuticular outgrowths (Figs. 3–7). On the ventral side of each pygypodium these appear as spurs whose points, when everted, are oriented towards the animal's abdomen. Dorsally, these outgrowths are represented by smaller bi-, tri-, and quadri-cuspid barbs (Fig. 5).

Each abdominal segment up to the eighth has the typical arrangement of dorsal longitudinal muscles, *i.e.*, bilateral bundles of parallel longitudinal fibers running from one intersegmental fold to the next (Snodgrass, 1935). In segments eight and nine, this arrangement of the longitudinal muscles is modified (Figs. 2b, c). First, the muscle bundles float freely in the body cavity, with only a few of the fibers attaching at the eighth-ninth intersegmental fold. Second, as the fibers to the dorsal pygypodia proceed posteriorly within the eighth segment, their orientation pattern abruptly changes from a parallel to a twisted one, giving the muscle bundles an appearance similar to twine or spun yarn (Fig. 2b). Within the ninth segment, the tangle resolves itself into a series of smaller bundles, each of which is inserted directly into the inside tip of a dorsal pygypodium. The shorter, stouter ventral pygypodia present a completely different picture of muscle attachment (Fig. 2c). There are six muscle bands, three of which originate from the left and three from the right antecosta of the eighth segment. The bands from the left side insert respectively on the left lateral and the two central pygypodia, while the mirrored or opposite arrangement occurs on the right side. Thus the two central pygypodia each receive two muscle bands, one from either side.

### *Fine structure*

The endocuticle of the integument appears vaguely lamellar because of the orientation of bundles of microfibrils (Fig. 8). The microfibrils form a continuous sheet which is relatively planar in the dorsal region of a pygypodium, but not in the ventral region, where the spurs are found. Here, the sheet forms a pattern of peaks and valleys (Fig. 8). The peaks are located beneath the spurs, while the valleys lie between them. Microfibrils from the peaks extend into the spurs while, conversely, microfibrils in





the valley portions appear to be attached to the epidermis by relatively densely staining material which may represent adhesion plaques. The endocuticle beneath the microfibril sheet appears somewhat amorphous (Figs. 8, 9).

The epidermal cells are irregular in shape, loosely organized, and have interlocking borders (Figs. 8, 9). These borders are marked by extensive desmosomes (Fig. 8), and individual cells present numerous vacuoles and small spherical objects at the epidermal-cuticular interface; these may represent Golgi complexes or plasma membrane plaques which may be involved in cellular uptake or secretion (Locke, 1976).

Figures 10 and 11 present thin sections of single pygypodial muscle bands. With our fixation, the connective tissue sheaths around the muscle bands appear rippled. Transverse sections show the typical actin-myosin arrangement within some of the fibrils of the muscle bands (Fig. 11). The fibrils are not all arranged in parallel; thus, in a single transverse plane, only a small proportion of the cut fibrils present the typical "cross-section" actin and myosin patterning. These sections also show variability in the arrangement of the Z-line material (Fig. 11).

Figure 11 (inset) shows the hemidesmosomal attachments of the Z-line material of the pygypodial muscle to the plasma membrane and the basement membrane.

### *Operation of the pygypodia*

*Inversion.* Inversion occurs when the contracting muscle fibers pull the pygypodial tip into the hollow of the pygypodium. This process diminishes the volume of the appendages and is therefore in opposition to the coelomic fluid pressure, which may contribute to the compression of the newly inverted integument. The dorsal crease may provide the pygypodium with an initial fold to guide the further packing of the integument and spurs. During this process, the latter are pried off the substrate, one row at a time, and are internalized and reoriented to fold properly and to intermesh compactly (Figs. 6, 7).

*Eversion.* There is no array of muscle fibers, comparable to those discussed above, whose action would directly evert the pygypodia. The lack of any obvious musculature for this purpose, together with the ease with which the pygypodia may be everted by increasing the coelomic pressure (as in pressing gently on the animal's abdomen), suggests that the eversion mechanism may be hydrostatic, and that the animal forces the podia to extend by tightening the abdominal musculature and driving hemolymph into them. During this process, the basement membranes of the inverted and everted portions of the pygypodia are in tight apposition (Fig. 9). This close contact produces a structure resembling a movable plug (the still-inverted portion of the pygypodium) and limits the amount of coelomic fluid in regions distal to the plug. As eversion proceeds, each successive transverse array of spurs arrives at the outer tip of the

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FIGURE 2a. Outline of tergum of the ninth abdominal segment (IX) and everted pygypodia, dorsal view. The pygypodial apparatus exhibits clear bilateral symmetry and all pygypodia originate from a common basal stalk which is covered by a brush of fine hairs. Only the dorsal podia are visible: the outer four on each side originate from a common branch of the main stalk, and the remaining two from a separate, more medial branch.

FIGURE 2b. Ventral view of the dorsal body wall of segments VII, VIII and IX, with the ventral pygypodia removed, showing the muscle arrangement and pleural plates (Pl). Note the abrupt change in the arrangement of the medial dorsal longitudinal muscles (\*) from a parallel to a twisted one and back again, as they approach the sites of insertion on the pygypodia. The lateral dorsal longitudinal muscles (\*\*) follow the more usual intersegmental course and are not connected to the pygypodia.

FIGURE 2c. As in Figure 2b, but with the ventral pygypodia still present. The muscles inserting on the ventral pygypodia (arrows) are probably those identified by Snodgrass (1935) as the paradorsal muscles. Some of the dorsal longitudinal muscles (\*, \*\*) seen in Figure 2b are represented here for reference.

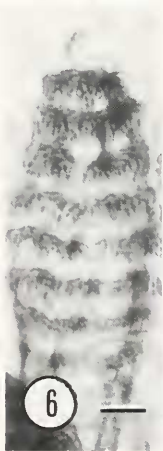
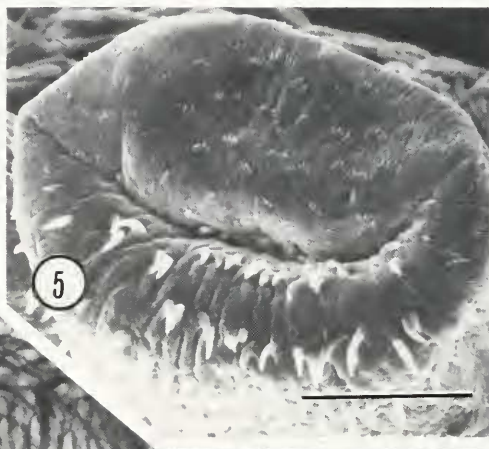
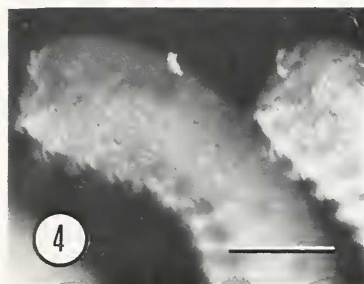
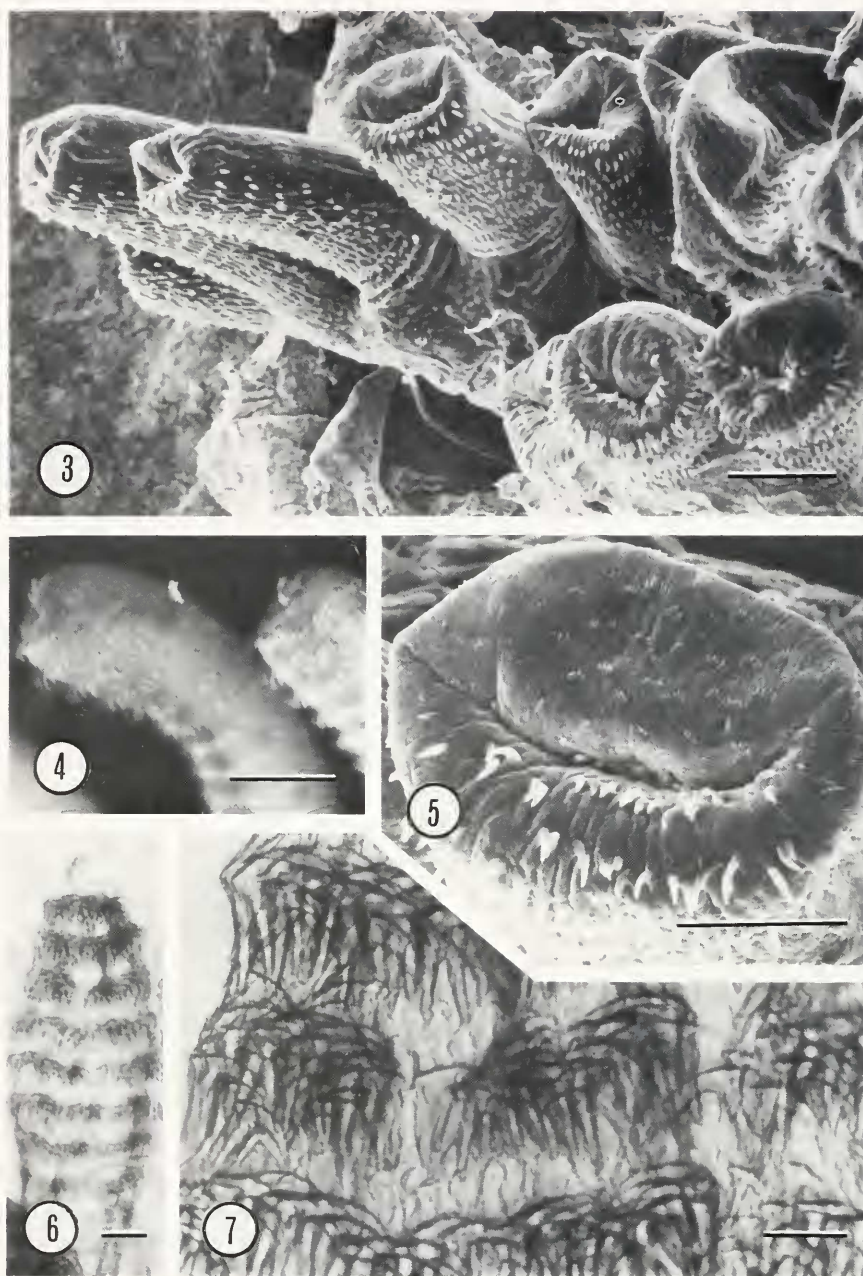


FIGURE 3. Scanning electron micrograph of part of the pygopodial array of a *Photuris* larva, from the postero-ventral aspect, showing several partially everted dorsal and two ventral pygopodia. Note the uniformity of the transverse rows of ventral spurs on each of the podia. Bar = 0.1 mm.

FIGURE 4. Light micrograph of the tips of two fully everted pygopodia. The terminal protrusion or bulb is visible on each, and some of the rows of ventral spurs may be seen along the sides. Bar = 0.1 mm.

FIGURE 5. Ventral pygopodium from the group in Figure 3, rotated to show a view directly "into" the partially everted pygopodium. Spurs which are completely external are erect in position, while those still within the inverted portion of the podium show the flattened, packed orientation they maintain inside.

unfolding pygypodium (Fig. 5) and each individual spur springs erect and perpendicular to the integument.

Depending on the nature of the substrate, a spur may either penetrate or, if the substrate is impenetrable, tip sideways, lying nearly flat and parallel to the integument. This effect is most readily observed in spurs which are not members of recently everted arrays.

*Walking.* As an ancillary appendage for walking, the basal stalk of the inverted pygypodium may be used like a suction cup. The area is always damp, and the presence of even a small amount of fluid results in adhesion to the substrate. The animal may also be held by surface molecular forces in the liquid film (Wigglesworth, 1972). Secretions, possibly mucus, from the terminal abdominal segments apparently supply necessary fluid, and the whole area is rich in tufts of bristles (Figs. 2a, b) which help hold and distribute the moisture. Once attachment is made to the substrate, the larva can use its abdominal musculature to move the body forward.

*Other functions.* Under special circumstances, *i.e.*, when the animal is trying to right itself or is exploring unusual territory (such as a glass slide), selected pygypodia partially invert, and their tips act as suction cups (Fig. 5); the ventral rings of spurs are turned upwards out of the way, and the center of the tip forms a depression by contraction of the internal muscle fibers. Release from the suction is accomplished by everting the pygypodium and having the bulb at the tip break the seal.

## DISCUSSION

Inversion or eversion of the pygypodia may be described as the result of antagonism between the podal muscles and those muscle groups which control the coelomic (or, more precisely, pseudocoelomic) pressure in the abdomen. The complex arrangement of the pygypodial muscles (and their presumably equally complex innervation) may be responsible for the high level of control exhibited during movement of individual pygypodia and of the cluster as a whole. The apparent difference between the dorsal longitudinal muscles of segment VII and the podal retractor muscles of segment VIII probably represents a modification of the typical arrangement of the larval abdominal musculature in this region.

In cross section, the pygypodial integument consists of an outer supporting ring of exo- and endo-cuticle encasing a flexible inner layer of epidermal cells and basement membrane. The cuticle, and in particular the ventral arrays of spurs, supports the cylindrical shape of the pygypodia. Totally inverted podia are compressed, yet they still remain semicylindrical. The series of transverse rings generated by the packing of the spurs supports and maintains the cylindrical shape of the inverted parts of a pygypodium, more or less in the fashion of the cartilage rings in the vertebrate trachea.

Within the endocuticle, the continuous sheet of parallel microfibrils forms a tough, elastic inner sheath, which strengthens the whole pygypodium. Thus, during eversion, the increased fluid pressure is evenly distributed throughout the hollow of the tube. There appear to be no irregularities or bulges in the integument, even when it is

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The small barb-like structures on the dorsal portion of the unfolding pygypodium are typically found on the dorsal regions of the pygypodia and are probably homologous to the ventral spurs. Bar = 0.05 mm.

FIGURE 6. Light micrograph of a fully inverted pygypodium which has been treated with potassium hydroxide to remove all cellular constituents. The compact packing of the internalized spurs is visible here. Bar = 0.03 mm.

FIGURE 7. As in Figure 6, but higher magnification. The compact "criss-cross" arrangement of the internalized spurs is clearly visible. Bar = 0.02 mm.



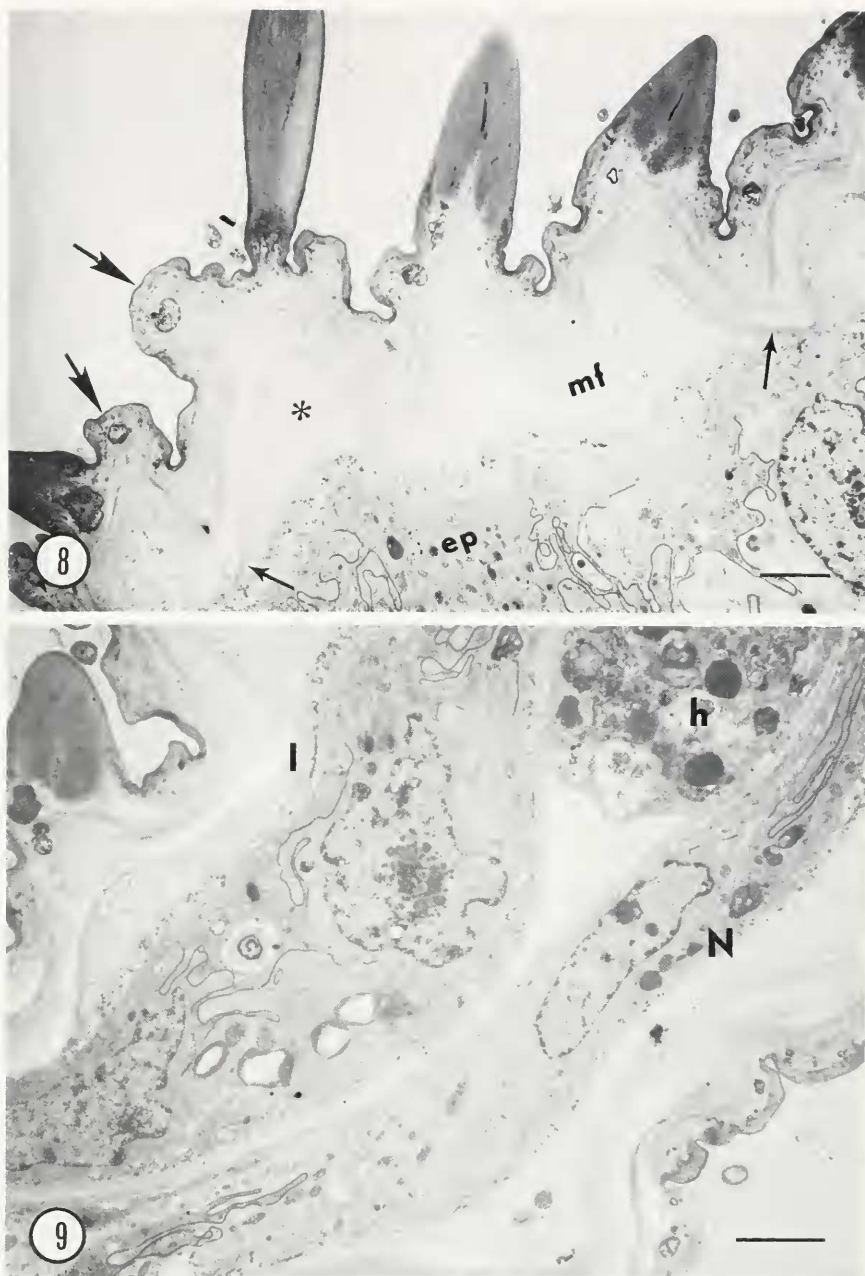


FIGURE 8. Transmission electron micrograph of transverse section through the pygopodial integument. The electron-dense spurs have broad outcroppings or folds of cuticle at their bases (large arrows) and the endocuticle shows the peak-and-valley patterning of the microfibril sheet (mf). Note the apparent attachment of the latter to the epidermis (ep; small arrow) as well as the extension of the fibrils into the spines. This view also shows the amorphous endocuticle (\*). Bar = 2  $\mu$ m.

FIGURE 9. TEM of part of the inverted (I) and non-inverted (N) walls of a partially retracted pygopodium. The basement membranes between the two integuments are closely apposed into a relatively tight seal which limits the space available for coelomic fluid between them. (The seal extends to and includes a hemocyte (h) which has apparently gotten caught in the gap.) The epithelial cells are irregular in shape, loosely organized, and have few sites of attachment to the basement membrane or to the cuticle. Bar = 2  $\mu$ m.



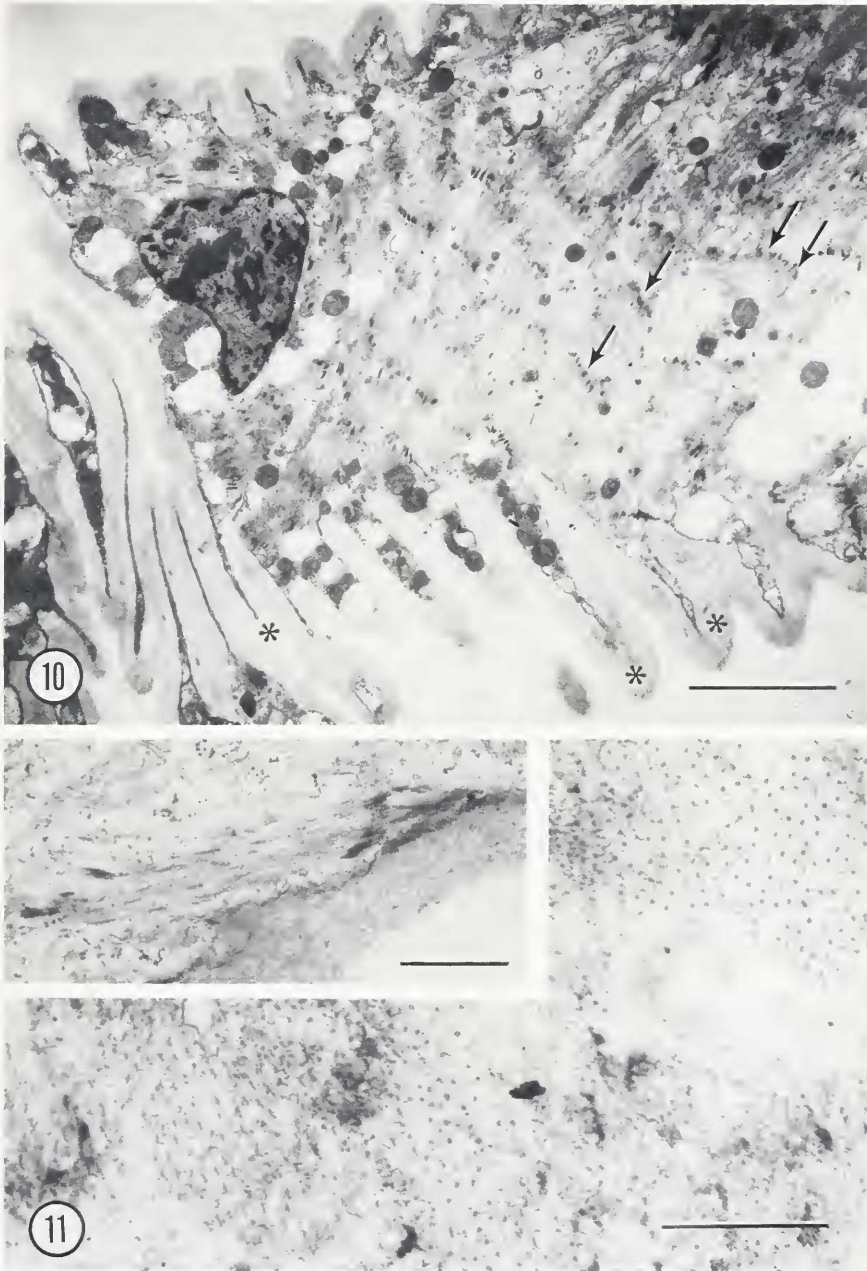


FIGURE 10. TEM of a longitudinal section of a contracted pygypodial muscle, showing the pleated appearance of the connective tissue sheath (\*) and the variability in the arrangement of the Z-line material (small arrows). Bar =  $0.33\ \mu\text{m}$ .

FIGURE 11. TEM of a transverse section through part of a pygypodial muscle fiber. Only a small portion (upper right) of the total area shown presents the typical cross-sectional actin-myosin filament patterning because these muscles have a more or less felt-like arrangement, with parts of the fibrils going in different directions. Bar =  $0.5\ \mu\text{m}$ . *Inset*. Site of attachment of Z-line material to the plasma membrane. Bar =  $0.5\ \mu\text{m}$ .

under pressure. If the animal's abdomen is pressed with forceps, fluid leaks from the intersegmental membranes long before the pygypodia are ruptured, which suggests that the integument of these appendages is built to withstand the pressure and stress that must be generated in any hydrostatic system.

The loose folds of the exocuticle and of the microfibril sheet of the endocuticle allow these layers the necessary "give" to accommodate the changes in circumference and shape that accompany pygypodial movement. The apparently unstructured inner layers of the cuticle have no such folds, and so they must either stretch (Filshie, 1982) or flow (Vincent, 1981) as the cuticle is stressed. The connective tissue sheaths of the muscles can apparently accommodate substantial changes in muscle length, and the loose organization and highly folded or involuted surfaces of the epidermal cells are characteristic of epithelia that must withstand changes in shape (Vincent, 1981). The sparse but regular attachments of the cells to the microfibril sheet apically, and to the basement membrane proximally, must limit the displacement of the various components when the system is stressed.

The basement membrane may also play a role in generating and maintaining the tight occlusive seal between the inner and the outer walls of the plug region of a partially everted pygypodium. The basement membranes of the inverted and everted sections conform closely to one another, and so ensure that coelomic pressure will be applied to the plug (the inverted portion of the pygypodium) rather than be dissipated by the leakage of fluid between the outer and inner walls. The loose organization of the epidermal cells and the large intercellular spaces may also allow the tissues to "give" where necessary, and at the same time provide cushioning between the layers.

During eversion and inversion, the presentation or folding of the spurs may depend on the elasticity of the endocuticular microfibril sheet which interconnects all the spurs at their bases. Once released from the confines of the outer sleeve of the pygypodium, the individual spurs of each transverse array reorient from their "packed" positions to erect ones. The interconnection of the spurs by the microfibril sheet may smooth and integrate this transition so that the spurs are presented in an orderly and consistent fashion.

During inversion, the integument and associated arrays of spurs are internalized by the contraction of the pygypodial muscles. The dorsal longitudinal crease serves as a guide for the proper inversion of the integument and folding and compacting of the spurs, possibly by altering the direction of stress in the cuticle. With the seal lost around the plug, coelomic fluid may enter the pygypodium and aid in compacting the already inverted portions.

Even though the spurs lie flat when a pygypodium is in contact with smooth and impenetrable surfaces, they automatically revert back to erect positions when it is removed from these surfaces. Each spur is apparently braced, and its base reinforced by the associated portions of the underlying microfibril sheet. The design of the sheet may concentrate coelomic fluid pressure on the bases of the spurs, an arrangement which maintains undisturbed spurs in the perpendicular position, while allowing enough flexibility for spurs under external pressure to "give" or reorient.

The pygypodia may have evolved as modifications of the intersegmental membranes of the last abdominal segment. The typical larval intersegmental structure consists of antecostal cuticular folds (Snodgrass, 1935). To arrive at something like the pygypodia, one need only postulate extension of one such fold into deep and distinct pouches. The original dorsal longitudinal muscle insertions are carried in with the pouches to become associated with what are now the tips of the inverted pygypodia. Correspondingly, the ventral pygypodia may have developed in a similar

manner. The observed anomalies, *i.e.*, the twisting of the dorsal musculature and the crossing over of some of the ventral muscles to the opposite side of the midline, remain unresolved puzzles requiring further work and thought.

#### ACKNOWLEDGMENTS

We wish to thank Dr. Steven Brown for much helpful discussion along the way, and for reviewing the manuscript, Mr. Ryland Loos for the diagrams, and Mr. Robert Speck for help with the final publication prints. Supported by NSF grant #PCM78-22924 to H.Gh.

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